Toxicity Test Report
Task 4a. Conduct toxicity tests on Mine Waste Samples from 3-5 Technologies

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Project: Butte, Montana Mine Sites Toxicity Tests
Sent to: Jim Lazorchak, ORD/NERL

Introduction

Water samples from four mine sites in the Butte, MT vicinity were shipped to the U.S. EPA Laboratory in Cincinnati, Ohio. A series of acute aquatic toxicity tests with *Pimephales promelas*, the fathead minnow, and *Ceriodaphnia dubia*, a freshwater invertebrate, and chronic aquatic toxicity tests with *Daphnia magna*, a freshwater invertebrate, were conducted on these samples. The purpose of these tests was to establish the level of toxicity for the discharge from the different mines sites and to evaluate the effectiveness of the treatment processes currently being used at these sites.

Definitions

Acute toxicity test: A test method that uses a short exposure period (i.e. 48 hours) to determine the lethal effects of an effluent or receiving water to a selected test organism.

Chronic toxicity test: A test method that uses an exposure period that is less than a complete life cycle (i.e. 4-7-days) to determine both the lethal and sub-lethal effects of an effluent or receiving water to a selected test organism. The sub-lethal effects can include growth or reproduction.

Definitive test: A test that uses a series of effluent or receiving water dilutions to determine the level of acute or short-term chronic toxicity a sample exhibits to a selected test animal.

Profile sample: A sample that is tested using only the 100% (undiluted) test sample.

No Observed Acute Effect Level (NOAEL): That concentration or percent sample in an acute test where the survival of the test animals is determined to not be statistically different from the survival of the control animals. If survival in the lowest test concentration is determined to be statistically different from the control, the data are evaluated to see if the survival in the lowest test concentration is greater than 40%. If it is, the assumption is made that the next dilution in the series would have survival not different from that of the control and this estimated data point is used as the NOAEL.

No Observed Effect Concentration: That concentration or percent sample in a short-term chronic test where the survival, growth or reproduction of the test animals is determined to not be statistically different from the survival, growth or reproduction of the control animals.

Fifty Percent Lethal Concentration (LC50): The estimated concentration of a compound or percent effluent or receiving water that would cause 50% mortality to the test animals.

Inhibition Concentration 25 (IC25): The estimated concentration of a compound or percent effluent or receiving water that would cause a 25% effect (reduction) in the reproduction or growth of the test animals.

Percent minimum significant difference (MSD%): The lowest percent difference between a control and effluent sample that an analysis of variance test (i.e. Dunnett's) can detect as being statistically different.

Methods

Samples were collected on Monday 7-7-99 and Tuesday 7-8-99 using a plastic graduated cup and 1 gallon cubitainers. Approximately 2 quarts were collected from each effluent, waste pile, reactor or receiving stream. After each sample was collected, air was depressed from each cubitianer, it was capped and placed in a cooler with 10 lbs of ice and shipped overnight to the EPA facility in Cincinnati. All coolers were received in good condition with all seals intact, and all samples were in acceptable condition. A total of six water samples collected from Calliope Mine were received on 7-7-99 and eight water samples collected from Crystal Mine, Lilly/Orphan Boy Mine and Peerless Mine were received on 7-8-99.

The most recent monthly chemical analyses were obtained from MSE and used to estimate what concentrations would be tested for each sample. Table 1 summarizes the chemistry of a selected number of metals used to determine the highest dilution to be tested and whether a Definitive test (dilution test) or Profile test (no dilution) would be performed. A zinc concentration of 150 µg/l was used as a guide to establish the highest dilution to run for both the definitive and profile tests. The acute toxicity of zinc is around 150 µg/l as long as the sample contains no complexing organics or high levels of carbonate.

Routine initial chemical parameters (Table 2) were determined and toxicity tests were started on arrival of the samples. The tests with *P. promelas* and *C. dubia* were 48-hr, renewed, acute tests, conducted at 20°C. Each sample was analyzed using both species, with the exception of Calliope Columns Out, which was analyzed using just the *C. dubia* acute test. In addition, nine samples were analyzed using a *D. magna* 4-day, growth and survival test, to provide a measure of the sensitivity of this method versus the two acute methods, as well as to provide a subsample of chronic test data for these sites. These included, Calliope Columns In, Influent Below Grade, Columns Out, Outflow Below Grade with treatment, Outflow Below Grade without Treatment, and Outflow Above Grade with pretreatment, Crystal Mine, Peerless Flow from Pile and Lilly Orphan Boy PT 3a.

All tests were conducted using moderately hard reconstituted water as the control and dilution water. Test conditions for *C. dubia, P. promelas*, and *D. magna* are contained in Tables 3, 4 and 5, respectively.

All LC50 values were determined using the 1994 U.S. EPA Toxicity Data Analysis Software that contains the Trimmed Spearman-Karber, version 1.5, which adjusts for control mortality. This software also was used to calculate the acute survival No Observed Acute Effect Level (NOAEL),

the chronic survival No Observed Effect Concentration (NOEC) and the chronic growth NOEC using Dunnett's, version 1.5, and the IC25 values using ICp version 2.0.

Results and Discussion

All tests were successfully completed with acceptable control survival (90% or greater) or growth (250 mg for fatheads and 10X the initial weight for *D. magna*) for all tests. *C. dubia* restarts were required for Calliope IBG and Lilly Orphan Boy Pt 3b, due to high mortality in the low test concentrations. In addition, the Peerless FIP *C. dubia* test should have been restarted, due to 100% mortality in the lowest test concentration, but insufficient test sample was left to rerun another dilution series. Fish restarts were required for Calliope IBG, Peerless FFP and Lilly Orphan Boy Pt 3b. The mortality of the fish in the high concentrations for these samples was not sufficient to determine an LC50 or an NOAEL. No *D. magna* tests were restarted. The results for the *C. dubia* tests are contained in Table 6, the fish results are contained in Table 7 and the *D. magna* results are contained in Table 8. Tables 9, 10, 11 and 12 contain summaries of all routine initial and final chemistries. Table 13 presents the chemical analyses of a select number of metals that were analyzed by ICP. A full lab report of the ICP analyses is also attached. **Note the concentrations measured represent the concentration at the time of the test not at time of collection.** A subsample was collected at the time the tests were run and acidified with Nitrex. The samples were then filtered before they were analyzed by ICP.

Calliope Results

Results from Calliope indicate both the Columns In (CI) sample and the Influent Below Grade (IBG) sample were toxic to all three species. For CI, the *C. dubia* had an LC50 value of 0.07%, with an NOAEL of 0.05%, the *P. promelas* LC50 was 0.99%, with an NOAEL of 0.625% and the *D. magna* had an IC25 value of 0.349%, with a growth NOEC <0.3125%. For IBG, the *C. dubia* LC50 was 0.23%, with an NOAEL of 0.05%, the *P. promelas* LC50 was 9.2%, with an NOAEL of 1.25% and the *D. magna* IC25 was 1.58%, with a growth NOEC of 2.5%. The results from the four profile (100% concentration only) samples (CO, R2, R3 and R4) for Calliope indicate they did not cause significant toxicity, or a reduction in growth, to the test animals. In fact, the growth of the *D. magna* in the four profile samples exceeded the growth of the *D. magna* in the control sample (Table 7). In summary, both CI and IBG were found to exhibit acute toxicity to *C. dubia* and *P. promelas* and chronic toxicity to *D. magna*. It was determined the four profile samples did not cause significant mortality to any species and in fact exposure to the profile samples enhanced the growth of the *D. magna*.

Using the NOAEL for *C. dubia* of 0.05% for the IBG sample, a predicted safe Zn concentration of 3.2 µg/l can be obtained, i.e. 0.0005 x 6,430 (Table 13) = 3.2. For the same sample, the NOAEL for *P. promelas* is 1.25%, which results in a safe predicted zinc level of 80.4 µg/l. An actual measured chronic level can be generated from the *D. magna* growth results, using the NOEC of 1.58% we find a safe Zinc level of 101.6 µg/l. So an acceptable clean up level should be between 3 and 101.6 µg/l. Looking at the effluent from each of the reactors then we find the following:

R2 acceptable zinc concentration is 590 µg/l (Table 13) for all species tested, which is a 90.8% reduction in zinc concentration from the influent concentration and 184 times higher than the predicted safe level (590 ÷ 3.2 = 184) NOAEL for *C. dubia*.

R3 acceptable zinc concentration is 1,230 µg/l for all species tested which is 80.9% reduction from the influent concentration and 384 times higher than the NOAEL calculated for *C. dubia*.

R4 acceptable zinc concentration is 1,370 µg/l for all species tested which is a 78.7% reduction from the influent concentration and 436 times higher than the NOAEL calculated for *C. dubia*.

An observation that can be drawn from these results is that there is a high amount of organic material that is binding or complexing the metals and not allowing them to be bioavailable.

For the column study the NOAEL for *C. dubia* in the Column influent (CI) would predict a safe zinc concentration of 133 µg/l (0.05% x 26,600 µg/l). There is virtually no zinc in the effluent from the column (CO) so the column study looks very promising. One recommendation is to run the column faster and see how much zinc comes through and rerun the toxicity tests as an evaluation tool.

Crystal Mine Results

The results from Crystal Mine show that *C. dubia* had an LC50 of 0.04%, with an NOAEL of 0.016%, the *P. promelas* had an LC50 of 0.37%, NOAEL of 0.25% and the *D. magna* had an IC25 of 0.25%, with a growth NOEC of 0.0313%. It should be noted the *C. dubia* NOAEL was estimated. Survival in the lowest test concentration, 0.0313%, was 70%, which was determined to be different from the control for survival. The spread of the data indicates that survival in a 0.016% sample would be 80% or greater, so this value is supplied as the NOAEL, but it should be used with caution.

Using the estimated NOAEL for *C. dubia* we predict a safe level for zinc from this effluent would be 8.5 µg/l (0.016 x 53,000 µg/l = 8.48). *D. magna* NOEC predicts an acceptable zinc concentration of 16.6 µg/l, which is about twice as high as the NOAEL for *C. dubia*. Since this zinc level is so low other metals like Cd might be adding to the toxicity. Cd was highest in the Crystal mine sample over any other mine by a factor of 10.

Peerless Mine Results

Results for Peerless Portal (PP) show the *C. dubia* LC50 was 1.2%, with an NOAEL of 0.78% and the *P. promleas* LC50 was 39.2% with an NOAEL of 12.5%. For Peerless Flow into Pile (FIP), the *C. dubia* LC50 and NOAEL were both less than 6.25%, due to 100% mortality in the lowest test concentration (6.25%). This test should have been restarted, but there was insufficient sample for a second test. The FIP results for *P. promelas* show an LC50 of 8.1% and an NOAEL of <6.25%, again below the lowest test concentration. All indications are this sample should have been tested using the concentration series used for PP. The results from Peerless Flow from Pile

(FFP) show a *C. dubia* LC50 of 1.6%, with an NOAEL of 0.625%. The *P. promelas* LC50 was 28.3%, with an NOAEL of 10% and the *D. magna* IC25 was 2.24%, with a growth NOEC of 1.25%. All samples were acutely toxic, with the *C. dubia* being more sensitive than the fish. Sample FFP also exhibited chronic toxicity to the *D. magna*, with survival and growth in one or more of the test concentrations being significantly different from the control.

Using the NOAEL for *C. dubia*, a safe zinc level for the Peerless Portal (PP) would be 13.1 µg/l (0.078 x 1680). Using the NOAEL for *C. dubia* for the flow from the pile to estimate a safe level for the flow into the pile as well, 47.3 µg/l was calculated for the flow from the pile and 31.8 µg/l was calculated for the flow into the pile.

Lilly Orphan Boy Results

The upstream profile sample for Lilly Orphan Boy had *C. dubia* and *P. promelas* LC50 and NOAEL values >100%. The downstream sample had a *C. dubia* LC50 of 33.9%, with an NOAEL of 25% and the *P. promelas* had an LC50 of 70.7% with and NOAEL of 50%. Two samples were collected at the Pt 3 discharge, Pt 3a and Pt 3b. For Pt 3a, the *C. dubia* LC50 was 0.34%, with an NOAEL of 0.25%, the *P. promleas* LC50 was 3.0%, with an NOAEL 1.0% and the *D. magna* IC25 was 0.653%, with a growth NOEC of 1.0%. For Pt 3b, the *C. dubia* LC50 was 0.24%, with an NOAEL of 0.125% and the *P. promelas* LC50 was 6.1%, with an NOAEL of 4.0%.

The upstream sample shows no toxicity to either species used, while the downstream sample was toxic to both species. Using the Downstream NOAEL for *C. dubia* a safe level for zinc would be 70 µg/l (0.25 x 280). For the portal samples, Pt3a and Pt3b, the C. dubia NOAEL's were 0.25% and 0.125% respectively. This would result in safe zinc levels of 38 µg/l and 18 µg/l. The low zinc values would tend to indicate that the alkalinity and hardness are low in both samples. Looking at Table 2, the results from the routine chemical analysis of the initial samples for Pt3a and Pt3b show hardness and alkalinity could not be measured and the downstream sample only has a hardness and alkalinity of 20 mg/l. Thus, very little complexing is available.

TABLE 1. Recent Chemical Analyses at Mines in the Butte Area Used to Determine Test Concentrations

	Metal Conc µg/l							
	Al	Cu	Zn	Mn	Pb	Type Of Test	Test Dilution % sample	Sample ID
Mine/location								
Calliope 05/05/99								
Influent Below Grade w/	14,100	3,050	11,100	3,700		Definit	5%	C2
Outflow Below Grade w/o	48	43	459	2,100		Definit	100%	C1
Above Grade pretreat	29	37	194	454		Definit	100%	C4
Below Grade with treat	14	8	249	661		Definit	100%	C3
Columns In								CI
Columns Out								CO
Peerless 04/02/99								
Portal	13	85	157	3,870	34	Definit	100%	PP
flow into Pile	12	8	551	36	34	Definit	100%	P1
flow from Pile		2,080	873	6,360	14,600	45 Definit	10%	P2
Lilly Orphan Boy 05/19/99								
PT3	3,100	142	15,700	6,050		Definit	4% & L2	L1
After Treat	24	2	17	3,360		Profile	100%	
Upstream	103	4	59	27	41	Profile	100%	L4
Downstream	157	7	324	124	41	Profile	100%	L3
Crystal Mine								
Mine Waste	22,000	25,700	80,600	13,700	400	Definit	0.5%	CM
Lime Treatment	1,590	4	20	4	40	Profile	100%	

TABLE 2. Arrival Chemistries

Sample	Temp (°C)	pH (S.U.)	Alkal. (ppm)	Hard. (ppm)	Cond. (µS/cm)	D.O. (ppm)
CI	7.9	2.97	N/A	540	1792	9.1
CO	7.1	7.96	282	940	1737	8.5
R3	7.1	8.02	86	180	428	8.2
IBG	7.9	3.62	N/A	720	483	9.7
R2	9.9	7.97	200	350	648	8.2
R4	6.8	7.84	198	480	990	8.9
CM	11.0	3.21	N/A	N/A*	1322	9.7
PP	9.6	6.98	42	150	276	9.7
FIP	10.3	4.08	N/A	360	372	9.8
FFP	9.6	6.58	40	160	380	9.8
PT 3b	9.5	3.49	N/A	N/A*	678	9.9
LOB Down	10.2	7.12	20	20	62	9.7
Pt 3a	9.7	3.40	N/A	N/A*	752	9.9
LOB Up	9.3	7.43	20	12	53	9.9
MHRW	23.5	7.95	60	96	344	8.4

* Hardness could not be determined due to a color interference.
Alkalinity values marked with N/A could not be determined due to low pH. pH> 4.7 required.
Samples and shortened designations.
Calliope Columns Out (CO)
Calliope Columns In (CI)
Calliope Outflow Below Grade w/o Treatment (R3)
Calliope Influent into treatment, below grade (IBG)
Calliope Below Grade w/treatment (R2)
Calliope Above Grade pretreatment Outflow (R4)
Crystal Mine (CM)
Peerless Portal (PP)
Peerless Flow into Pile (FIP)
Peerless Flow from Pile (FFP
Lilly Orphan Boy Pt 3b (Pt 3b)

Lilly Orphan Boy Downstream (LOB Down)
Lilly Orphan Boy Pt 3a (Pt 3a)
Lilly Orphan Boy Upstream (LOB Up)

TABLE 3. Standard Operating Procedures for *Ceriodaphnia dubia* acute toxicity tests for Superfund samples.

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	48 hr
Temperature	20°C ± 1°C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	30 ml (plastic cups)
Test Solution Volume	20 ml
Renewal of Test solution	Daily
Age of Test Organisms	Less than 24-hr-old
Number of Organisms/ per test chamber	5
Number of Replicate Chambers/Conc.	4
Number of Organisms/ Concentration	20
Feeding	none, fed while holding prior to test setup
Dilution Water	Moderately Hard Reconstituted Water
Endpoint	Mortality, LC50
Test Acceptability	≥ 90% survival in the controls

TABLE 4. Standard Operating Procedures for *Pimephales promelas* acute toxicity tests for Superfund samples.

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	48 hr
Temperature	20°C ± 1°C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	175 ml (plastic cups)
Test Solution Volume	150 ml
Renewal of Test-solution	Daily
Age of Test Organisms	3 to 7 days ± 24 hr age range
Number of Organisms/ per test chamber	10
Number of Replicate-Chambers/Conc.	2
Number of Organisms/ Concentration	20
Feeding	Feed newly hatched brine shrimp prior to testing. Do not feed during the test.
Dilution Water	Moderately Hard Reconstituted Water
Endpoint	Mortality, LC50
Test Acceptability	≥ 90% survival in the controls

TABLE 5. Standard Operating Procedures for *D. magna* Survival and Growth Toxicity Tests for Superfund Samples.

TEST PARAMETER	CONDITION
Test Type	static-renewal
Test Duration	4 days
Temperature	25 ^{0}C (±1 ^{0}C)
Photoperiod	16 h light: 8 h dark
Test Chamber Size	60 ml
Test Solution Volume	50 ml
Renewal of Test Solution	daily
Age of Test Organisms	<24 hours old
No. Organisms/Test Chamber	5
No. Replicate Test Chambers	4
No. Organisms/concentration	20
Feeding Regime	0.3 ml algae and 0.2 ml alfalafa
Test Solution Aeration	None
Dilution Water	Moderately Hard Water
Endpoint	Survival and Mean Dry Weight
Test Acceptability	90% or greater control survival control growth 10X initial weight

TABLE 6. Results from toxicity tests with *Ceriodaphnia dubia.*

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Calliope	Control	20/20	0.23	0.18-0.30	0.05	19
IBG	0.0125	18/20				
	0.025	19/20				
	0.05	18/20				
	0.1	15/20				
	0.2	16/20				
	0.4	7/20				
	0.8	0/20				
Calliope	Control	19/20	0.07	0.06-0.07	0.05	14
Columns	0.0125	19/20				
In	0.025	20/20				
	0.05	16/20				
	0.1	1/20				
	0.2	0/20				
Calliope	Control	19/20	N/A	N/A		
Profiles	R2	20/20			>100%	
	R3	19/20			>100%	
	R4	19/20			>100%	
	CO	16/20			>100%	
Crystal	Control	20/20	0.04	0.03-0.05	0.015625[A]	21
Mine	0.0313	14/20				
	0.0625	1/20				
	0.125	0/20				
	0.25	0/20				
	0.5	0/20				

A) Estimated value, low concentration of 0.03125% was different from the control for survival.

TABLE 6. Results from toxicity tests with *Ceriodaphnia dubia,* cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Peerless	Control	20/20	<6.25	N/A	<6.25	N/A
FIP	6.25	0/20				
	12.5	0/20				
	25	0/20				
	50	0/20				
	100	0/20				
Peerless	Control	20/20	1.6	1.3-1.8	0.625	4
FFP	0.625	19/20				
	1.25	16/20				
	2.5	0/20				
	5	0/20				
	10	0/20				
Peerless	Control	20/20	1.2	1.1-1.2	0.78	10
Portal	0.78	19/20				
	1.57	1/20				
	3.125	0/20				
	6.25	0/20				
	12.5	0/20				
Lilly Orphan	Control	19/20	0.34	No Limits	0.25	8
Boy Pt 3a	0.25	17/20				
	0.5	0/20				
	1	0/20				
	2	0/20				
	4	0/20				

TABLE 6. Results from toxicity tests with *Ceriodaphnia dubia,* cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Lilly Orphan	Control	20/20	0.24	0.21-0.28	0.125	11
Boy Pt 3b	0.0313	20/20				
	0.0625	17/20				
	0.125	19/20				
	0.25	9/20				
	0.5	0/20				
Lilly Orphan	Control	19/20	33.9	30.1-38.3	25	18
Boy	6.25	18/20				
Downstream	12.5	18/20				
	25	17/20				
	50	1/20				
	100	0/20				
Lilly Orphan	Control	19/20	N/A	N/A	>100%	p=0.171
Boy	100%	16/20				
Upstream						

TABLE 7. Results from toxicity tests with *Pimephales promelas.*

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Calliope	Control	20/20	9.2	6.8-12.4	1.25	21
IBG	1.25	20/20				
	2.5	13/20				
	5	15/20				
	10	10/20				
	20	0/20				
Calliope	Control	20/20	0.99	0.72-1.35	0.625	20
Columns	0.3125	17/20				
In	0.625	12/20				
	1.25	11/20				
	2.5	1/20				
	5	0/20				
Calliope	Control	20/20	N/A	N/A		
Profiles	R2	20/20			>100%	
	R3	20/20			>100%	
	R4	19/20			>100%	
Crystal	Control	20/20	0.37	0.24-0.46	0.25	24
Mine	0.0313	20/20				
	0.0625	20/20				
	0.125	19/20				
	0.25	15/20				
	0.5	6/20				

TABLE 7. Results from toxicity tests with *Pimephales promelas,* cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Peerless	Control	20/20	N/A*	N/A*	5	19
FFP	0.625	20/20				
	1.25	20/20				
	2.5	20/20				
	5	17/20				
	10	12/20				
restart 7-12	10	16/20	28.3	22.0-36.4	10	
	20	14/20				
	40	4/20				
Peerless	Control	20/20	39.2	31.9-48.3	12.5	14
Portal	6.25	20/20				
	12.5	19/20				
	25	16/20				
	50	8/20				
	100	0/20				
Lilly	Control	20/20	3.0	2.5-3.7	1	9
Boy Pt 3a	0.25	20/20				
	0.5	20/20				
	1	18/20				
	2	16/20				
	4	6/20				
Peerless	Control	20/20	8.1	7.0-9.4	<6.25	40
FIP	6.25	14/20				
	12.5	2/20				
	25	0/20				
	50	0/20				
	100	0/20				

* No LC50 (or Limits) could be generated from the first test. LC50 results based on data from second test with this sample.

TABLE 7. Results from toxicity tests with *Pimephales promelas,* cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Lilly Orphan	Control	20/20	6.1	5.1-7.3	4	17
Boy Pt 3b	4	17/20				
	8	5/20				
	16	0/20				
Lilly Orphan	Control	20/20	70.7	No Limits	50	26
Boy	6.25	20/20				
Downstream	12.5	20/20				
	25	20/20				
	50	20/20				
	100	0/20				
Lilly Orphan	Control	20/20	N/A	N/A	100%	p=1
Boy	100	20/20				
Upstream						

TABLE 8. Results from toxicity tests with *Daphnia magna*.

Sample	Conc.(%)	Survival (%)	$\bar{X}$ Wt.	CV %	Sur NOEC	Grw NOEC	MSD %	IC25
Calliope	Control	100	124	8.9	1.25	2.5	10	1.578
IBG	0.3125	100	115	8.7				
	0.625	100	116	2.6				
	1.25	100	116	2.6				
	2.5	45	28	64.3				
	5	0	0	0				
Calliope	Control	95	179	7.8	0.3125	<0.3125	12	0.349
Columns	0.3125	95	150	12.0				
In	0.625	10	11	200				
	1.25	0	0	0				
	2.5	0	0	0				
	5	0	0	0				
Profiles	Control	100	130	4.9	N/A	N/A		N/A
	R2	100	188	5.1				
	R3	100	154	5.2				
	R4	100	204	5.6				
Crystal	Control	100	152	9.9	0.25	0.03125	17	0.071
Mine	0.0313	100	131	8.4				
	0.0625	95	118	18.6				
	0.125	100	89	19.1				
	0.25	90	39	15.4				
	0.5	0	0	0				

TABLE 8. Results from toxicity tests with *Daphnia magna*.

Sample	Conc.(%)	Survival (%)	$\bar{X}$ Wt.	CV%	Sur NOEC	Grw NOEC	MSD %	IC25
Peerless	Control	100	140	14.0	1.25	1.25	11	2.24
Flow from	0.625	100	130	8.5				
Pile	1.25	100	128	6.3				
	2.5	85	98	17.3				
	5	40	24	62.5				
	10	0	0	0				
Lilly	Control	100	126	2.4	1	<0.25	7	0.653
Orphan	.25	100	116	3.4				
Boy Pt 3a	.5	95	105	4.8				
	1	80	70	11.4				
	2	15	30	86.7				
	4	0	0	0				

TABLE 9. Initial routine chemistries for *C. dubia, D. magna* and *P. promelas* tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
Cont.	0	8.11	7.97	8.5	8.7	348	347	19.5	20.1
CI	0.013	7.44	7.91	8.3	8.5	347	349	19.5	20.2
	0.025	7.38	7.90	8.3	8.6	347	378	19.5	20.1
	0.05	7.35	7.88	8.2	8.6	346	349	19.6	20.1
	0.1	7.36	7.86	8.2	8.6	343	349	19.7	20.1
	0.2	7.40	7.87	8.3	8.6	346	353	19.5	20.0
	0.313	8.00	7.96	8.6	8.7	346	347	20.5	21.1
	0.625	7.84	7.94	8.7	8.6	349	349	20.5	21.0
	1.25	7.84	7.83	8.6	8.6	353	353	20.4	21.1
	2.5	7.35	7.57	8.6	8.6	362	361	20.4	21.1
	5	7.13	6.64	8.6	8.6	381	408	20.3	21.0
IBG	0.013	7.27	7.89	8.3	8.5	348	352	19.6	20.1
	0.025	7.25	7.88	8.3	8.6	348	347	19.8	20.1
	0.05	7.23	7.87	8.3	8.6	346	347	19.6	20.2
	0.1	7.24	7.92	8.2	8.6	343	346	19.7	20.2
	0.2	7.34	7.94	8.3	8.6	344	348	19.7	20.2
	0.4	7.25	7.98	8.7	8.5	344	345	20.1	19.1
	1.25	8.04	7.98	8.6	8.6	342	343	20.2	20.9
	2.5	7.99	7.88	8.6	8.6	341	342	20.2	21.0
	5	7.76	7.87	8.6	8.5	347	295	20.2	21.1
	10	7.55	7.70	8.7	8.4	344	296	20.1	21.0
	20	7.24	7.28	8.7	8.6	340	298	20.0	21.0
CO	100	7.96	8.14	8.5	8.9	1737	1725	20.7	19.5
R2	100	7.97	7.76	8.0	8.8	648	643	20.9	19.4
R3	100	8.02	8.07	8.2	8.7	428	426	20.9	19.4
R4	100	7.84	7.93	8.9	8.7	990	979	20.9	19.4

TABLE 9. Initial routine chemistries for *C. dubia, D. magna* and *P. promelas* tests. (cont'd)

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
CM	0.0313	7.94	8.12	8.4	8.5	345	347	19.3	20.9
	0.0625	8.04	8.12	8.4	8.6	345	347	19.1	21.0
	0.125	8.04	8.07	8.4	8.6	345	347	19.1	20.8
	0.25	8.01	8.02	8.4	8.5	346	348	19.1	21.0
	0.5	7.84	7.90	8.4	8.5	349	349	19.1	21.1
PP	0.78	7.84	7.84	8.5	8.6	353	349	20.4	20.1
	1.56	7.81	7.85	8.5	8.6	346	343	20.3	20.0
	3.125	7.85	7.82	8.5	8.6	347	347	20.4	20.0
	6.25	7.85	7.69	8.6	8.6	355	348	20.5	20.0
	12.5	7.69	7.52	8.5	8.6	348	350	20.4	19.9
	25	7.51	7.44	8.8	8.6	333	352	19.7	20.1
	50	7.17	7.16	9.0	8.6	310	315	19.4	20.3
	100	6.58	N/A	9.2	N/A	276	N/A	19.6	20.2
FIP	6.25	7.81	7.75	8.7	8.5	348	344	19.0	20.1
	12.5	7.68	7.71	8.7	8.5	342	341	19.1	20.2
	25	7.44	7.56	8.7	8.7	339	335	19.1	20.1
	50	7.04	7.25	8.8	8.9	329	323	19.1	20.0
	100	3.84	4.04	9.1	9.8	374	373	19.0	20.1
FFP	0.625	8.05	8.01	8.6	8.6	345	347	19.3	19.4
	1.25	8.06	8.02	8.6	8.6	347	347	19.2	19.3
	2.5	8.04	8.00	8.6	8.6	346	347	19.3	19.2
	5	7.96	7.97	8.6	8.7	350	348	19.3	19.2
	10	7.79	7.85	8.6	8.8	348	348	19.2	19.2

TABLE 9. Initial routine chemistries for *C. dubia*, *D. magna* and *P. promelas* tests. (cont'd)

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
Pt 3b	0.0313	8.07	7.81	8.7	8.3	351	335	20.0	20.0
	0.0625	8.02	7.84	8.7	8.3	349	339	19.8	19.9
	0.125	8.02	7.83	8.7	8.3	349	338	19.8	19.7
	0.25	8.02	7.90	8.7	8.3	347	335	19.6	19.8
	0.5	7.98	7.57	8.7	8.3	346	337	19.8	20.0
	4	7.75	7.85	8.6	8.4	354	309	19.8	21.1
	8	7.53	7.50	8.6	8.5	356	310	19.8	21.2
	16	7.23	7.04	8.7	8.5	363	324	19.7	21.2
LOB	6.25	8.05	8.04	8.7	8.4	329	330	20.6	19.5
Down	12.5	8.01	8.04	8.7	8.4	312	313	20.2	18.5
	25	7.94	8.00	8.7	8.4	280	277	20.4	19.5
	50	7.71	7.89	9.1	8.6	211	203	19.8	19.3
	100	7.06	7.11	9.6	8.9	55	50	19.0	19.3
Pt 3a	0.25	8.02	8.01	8.4	8.5	347	348	19.1	20.1
	0.5	8.04	8.07	8.4	8.5	346	348	19.2	19.9
	1	8.03	7.97	8.4	8.5	346	348	19.2	20.0
	2	7.97	7.96	8.4	8.5	347	349	19.3	19.9
	4	7.84	7.81	8.4	8.5	349	351	19.3	20.0
LOB Up	100	6.56	7.03	9.3	10.2	51	43	20.3	19.2

TABLE 10. Final routine chemistries from *C. dubia* tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
CI	cnt	8.13	8.09	8.7	8.5	356	351	20.7	20.8
	0.013	8.13	8.09	8.7	8.6	356	352	20.8	20.9
	0.025	8.14	8.11	8.7	8.6	354	352	20.9	20.7
	0.05	8.13	8.11	8.7	8.7	353	351	21.0	20.8
	0.1	8.12	8.11	8.7	8.6	350	351	20.9	20.6
	0.2	8.13	8.11	8.8	8.6	349	354	20.8	20.6
IBG	cnt	8.07	8.08	8.7	8.7	341	353	20.6	20.5
	0.013	8.03	8.08	8.7	8.7	355	353	20.7	20.6
	0.025	8.03	8.09	8.7	8.8	351	350	20.8	20.9
	0.05	8.02	8.09	8.7	8.7	348	349	20.8	20.8
	.1	8.04	8.09	8.7	8.7	345	348	20.8	20.7
	.2	8.04	8.09	8.7	8.8	346	347	20.8	20.7
	.4	8.10	8.12	8.7	8.7	347	352	20.2	21.0
	.8	8.11	8.15	8.5	8.6	347	353	20.2	21.0
Profile	cnt	7.97	8.02	8.6	8.7	345	351	20.4	21.0
	CO	8.45	8.41	8.6	8.5	1659	1648	20.5	21.0
	R2	8.17	8.18	5.0	7.9	626	643	20.5	20.9
	R3	8.15	8.07	8.7	8.5	454	424	20.5	20.8
	R4	8.11	8.24	7.3	8.2	957	957	20.5	21.0
CM	cnt	8.09	8.17	8.5	9.0	354	348	20.8	21.0
	0.0313	8.09	8.16	8.6	8.9	356	348	20.6	21.0
	0.0625	8.10	8.17	8.6	8.9	356	348	20.6	20.9
	0.125	8.10	8.16	8.6	8.9	358	349	20.6	20.8
	0.25	8.10	8.15	8.6	8.9	359	349	20.6	20.9
	0.5	8.00	8.12	8.6	8.9	359	350	20.6	21.0

TABLE 10. Final routine chemistries from *C. dubia* tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
PP	cnt	8.10	8.09	8.6	8.6	346	351	20.5	20.6
	0.78	8.02	8.08	8.6	8.6	348	350	20.7	20.5
	1.57	8.03	8.09	8.6	8.6	346	350	20.8	20.8
	3.125	7.99	8.07	8.6	8.6	347	350	20.8	20.7
	6.25	8.02	8.03	8.6	8.6	347	346	20.8	20.7
	12.5	7.96	8.00	8.6	8.6	346	353	20.7	20.7
FIP	cnt	8.12	N/A	8.6	N/A	352	N/A	20.5	N/A
	6.25	8.09	N/A	8.7	N/A	354	N/A	20.5	N/A
	12.5	8.02	N/A	8.7	N/A	353	N/A	20.6	N/A
	25	7.81	N/A	8.7	N/A	347	N/A	20.6	N/A
	50	7.60	N/A	8.7	N/A	337	N/A	20.6	N/A
	100	4.16	N/A	8.7	N/A	353	N/A	20.6	N/A
FFP	cnt	8.15	8.11	8.5	8.7	358	341	20.7	21.0
	0.625	8.13	8.12	8.6	8.7	356	350	20.7	21.0
	1.25	8.13	8.10	8.6	8.7	360	351	20.6	21.0
	2.5	8.12	8.09	8.7	8.7	358	351	20.7	20.9
	5	8.11	8.07	8.7	8.7	359	351	20.7	21.0
	10	8.08	8.02	8.6	8.7	358	352	20.9	20.8
Pt 3b	cnt	8.15	8.14	8.4	8.4	348	350	20.8	20.3
	0.0313	8.10	8.09	8.5	8.5	358	357	20.8	20.2
	0.0625	8.12	8.11	8.5	8.4	357	358	21.0	20.2
	.125	8.09	8.08	8.5	8.4	356	356	21.0	20.1
	.25	8.11	8.09	8.4	8.4	354	353	21.0	20.2
	.5	8.10	8.10	8.5	8.4	359	358	20.9	20.1

TABLE 10. Final routine chemistries from *C. dubia* tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
LOB	cnt	8.27	8.19	8.6	8.9	359	351	20.6	20.8
Down	6.25	8.24	8.16	8.6	8.8	338	333	20.8	20.7
	12.5	8.21	8.16	8.6	8.9	323	315	20.6	20.7
	25	8.10	8.11	8.6	8.9	290	280	20.6	20.7
	50	8.02	8.03	8.6	8.9	227	207	20.8	20.7
	100	7.80	7.59	8.6	8.9	72	60	20.7	20.7
Pt 3a	cnt	8.16	8.15	8.7	8.9	353	352	20.2	21.0
	0.25	8.13	8.16	8.7	9.0	356	353	20.1	20.9
	.5	8.12	8.17	8.8	8.9	360	355	20.3	20.9
	1	8.10	8.17	8.8	9.0	356	353	20.2	20.8
	2	8.07	8.14	8.8	8.9	358	353	20.2	20.8
	4	8.03	8.12	8.8	9.0	360	354	20.2	20.8
LOB	cnt	8.09	8.24	8.8	8.8	359	352	20.0	20.8
Up	100%	7.43	7.57	8.8	8.9	61	52	20.4	20.8

TABLE 11. Final routine chemistries from *P. promelas* toxicity tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
IBG	cnt	7.98	7.96	8.7	8.5	352	355	20.3	20.7
	1.25	8.06	8.10	8.7	8.5	354	357	20.3	20.4
	2.5	8.06	8.09	8.8	8.5	355	348	20.3	20.5
	5	8.00	7.97	8.5	8.7	350	354	20.5	20.5
	10	7.94	7.89	8.5	8.8	352	355	20.6	20.5
	20	7.76	7.65	8.5	8.8	344	350	20.6	20.5
CI	cnt	7.89	8.00	8.5	8.6	350	350	20.7	19.9
	0.313	7.94	8.05	8.5	8.7	356	372	20.6	20.2
	0.625	7.96	8.05	8.6	8.8	357	357	20.6	20.5
	1.25	7.94	8.00	8.6	8.8	360	362	20.8	20.5
	2.5	7.81	8.00	8.6	8.7	370	394	20.7	20.5
	5	7.60	N/A	8.6	N/A	387	N/A	20.8	N/A
Profile	cnt	7.96	8.04	8.7	8.6	347	352	20.5	20.5
	R2	7.84	7.97	8.4	8.4	424	430	20.5	20.5
	R3	8.01	8.16	4.4	6.9	625	642	20.4	20.5
	R4	8.00	8.23	6.4	7.6	950	978	20.4	20.5
CM	cnt	7.88	8.09	8.7	8.8	354	357	20.4	20.4
	0.0313	7.97	8.06	8.5	8.6	352	350	20.6	20.6
	0.0625	7.99	8.07	8.6	8.8	352	349	20.7	20.6
	0.125	7.97	8.08	8.6	8.8	353	351	20.5	20.7
	0.25	7.96	8.11	8.7	8.8	355	351	20.4	20.6
	0.5	7.95	8.09	8.7	8.8	356	351	20.4	20.6

TABLE 11. Final routine chemistries from *P. promelas* toxicity tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
PP	cnt	8.08	8.07	8.5	8.6	356	346	20.5	20.6
	6.25	7.96	8.05	8.5	8.7	356	360	20.5	20.5
	12.5	7.89	7.97	8.5	8.7	349	348	20.5	20.6
	25	7.86	7.92	8.6	8.7	341	341	20.5	20.6
	50	7.74	7.79	8.6	8.7	317	323	20.5	20.6
	100	7.48	N/A	8.5	N/A	355	N/A	20.3	N/A
FIP	cnt	7.84	8.07	8.6	8.7	358	353	20.3	20.4
	6.25	7.86	8.05	8.7	8.9	358	352	20.3	20.4
	12.5	7.82	8.01	8.7	9.0	354	345	20.3	20.4
	25	7.67	N/A	8.8	N/A	359	N/A	20.2	N/A
	50	7.49	N/A	8.9	N/A	356	N/A	20.2	N/A
	100	4.09	N/A	8.9	N/A	371	N/A	20.2	N/A
FFP	cnt	8.02	8.04	8.5	8.7	388	352	20.2	20.6
	0.625	7.98	8.08	8.5	8.7	354	353	20.3	20.5
	1.25	7.96	8.04	8.6	8.7	355	350	20.3	20.5
	2.5	7.93	8.04	8.6	8.7	353	351	20.3	20.5
	5	7.94	8.01	8.6	8.7	359	354	20.3	20.5
	10	7.85	8.00	8.6	8.7	357	353	20.3	20.6
	10	7.87	7.87	8.3	8.6	356	349	20.5	20.6
	20	7.82	7.80	8.3	8.6	357	353	20.6	20.5
	40	7.69	7.70	8.3	8.6	357	359	20.6	20.4
Pt 3b	cnt	7.88	7.81	8.2	8.5	355	338	20.6	20.5
	4	7.88	7.84	8.3	8.7	358	351	20.4	20.5
	8	7.85	7.79	8.4	8.7	360	359	20.6	20.5
	16	7.60	7.56	8.5	8.7	367	365	20.5	20.5

TABLE 11. Final routine chemistries from *P. promelas* toxicity tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
LOB	cnt	8.01	8.10	8.7	8.6	369	370	19.5	20.7
Down	6.25	8.04	8.08	8.7	8.7	340	344	20.4	20.6
	12.5	8.01	8.06	8.8	8.7	318	327	20.3	20.6
	25	7.97	8.01	8.7	8.6	289	318	20.2	20.6
	50	7.92	7.99	8.6	8.7	217	266	20.4	20.7
	100	7.61	N/A	8.7	N/A	60	N/A	20.5	N/A
Pt 3a	cnt	7.97	7.97	8.5	8.6	355	358	20.2	20.6
	0.25	7.96	8.03	8.5	8.8	353	350	20.4	20.5
	0.5	7.97	8.02	8.6	8.7	358	351	20.4	20.5
	1	7.94	8.03	8.5	8.7	357	351	20.3	20.5
	2	7.92	8.00	8.6	8.7	360	356	20.3	20.6
	4	7.88	7.98	8.6	8.7	359	355	20.4	20.7
LOB	cnt	8.13	8.11	8.6	8.7	351	348	20.4	20.6
Up	100	7.65	7.77	8.7	8.7	52	46	20.3	20.6

TABLE 12. Final routine chemistries from *D. magna* toxicity tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	low	high	low	high	low	high	low	high
CI	cnt	7.90	8.15	7.8	8.4	356	361	24.0	24.6
	0.313	7.85	7.97	7.8	8.0	351	362	24.2	24,6
	0.625	7.90	8.14	8.0	8.4	357	361	24.2	24.9
	1.25	7.91	7.95	8.0	8.0	366	366	24.1	24.8
	2.5	N/A	7.88	N/A	8.0	N/A	375	N/A	24.2
	5	N/A	7.42	N/A	8.2	N/A	421	N/A	24.3
IBG	cnt	7.65	8.05	7.7	8.1	357	369	24.2	24.5
	0.313	7.67	8.11	7.7	8.1	353	359	24.3	24.6
	0.625	7.64	8.07	7.6	8.1	353	359	24.4	24.5
	1.25	7.69	8.01	7.6	8.1	354	357	24.1	24.5
	2.5	7.80	8.04	7.9	8.1	353	356	24.3	24.5
	5	7.82	7.97	8.0	8.1	351	368	24.1	24.5
Profile	cnt	7.77	8.02	7.6	8.2	357	366	24.1	24.7
	R2	8.07	8.23	7.0	7.6	428	443	24.2	24.6
	R3	7.74	7.98	7.5	7.9	629	639	24.1	24.6
	R4	8.14	8.27	7.3	7.8	946	969	24.2	24.6
CM	cnt	7.83	8.31	7.9	8.3	350	360	24.4	24.8
	.0313	7.85	8.22	7.8	8.3	353	357	24.4	24.6
	.0625	7.85	8.10	7.8	8.2	351	359	24.2	24.6
	.125	7.88	8.06	8.0	8.0	355	363	24.1	24.5
	.25	7.91	8.02	8.0	8.0	353	357	24.0	24.6
	.5	7.94	8.00	8.0	8.0	356	359	24.6	24.6

TABLE 12. Final routine chemistries from *D. magna* toxicity tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	low	high	low	high	low	high	low	high
FFP	cnt	7.87	8.02	7.8	8.3	353	368	24.2	24.6
	0.625	7.86	8.00	7.7	8.3	352	362	24.3	24.7
	1.25	7.82	7.97	7.7	8.2	351	366	24.3	24.8
	2.5	7.82	7.96	7.7	8.2	352	361	24.2	24.8
	5	7.83	8.03	8.0	8.2	354	364	24.5	24.9
	10	7.82	8.00	8.1	8.1	354	372	24.3	24.4
PT 3a	cnt	7.86	8.13	7.7	8.5	353	366	24.4	24.6
	0.25	7.84	8.12	7.8	8.5	354	358	24.3	24.6
	0.5	7.88	8.10	7.9	8.5	350	360	24.3	24.5
	1	7.81	8.08	7.7	8.4	351	358	24.2	24.5
	2	7.84	8.05	7.6	8.4	352	365	24.2	24.5
	4	7.92	7.96	8.2	8.5	352	364	24.2	24.5

TABLE 13. Chemical Analyses of Samples for Selected Metals
Concentration represents the sample at the time toxicity tests were run, not at time of collection.

Concentration in µg/l:Filtered Samples

	Al	Cd	Cu	Zn	Mn	Pb
Lilly Orphan Boy						
L1 PT3a	7,210	189	310	15,200	5,580	130
L2 downstream	121	17	4	280	201	<4
L4 upstream	84	<1	2	16	611	<4
L3 PT3b	6,820	180	300	14,700	5,620	121
Peerless						
PP Portal	294	12	195	1,680	4,390	23
P1 Flow into Pile	1,390	36	760	5,090	9,070	48
P2 Flow out of Pile	757	35	121	5,470	10,100	161
Calliope						
C1 Reactor 3 outflow	69	14	26	1,230	1,640	<4
C2 Influent to reactors	6,620	26	1,570	6,430	2,560	9
C3 Reactor 2 outflow	44	2	28	590	1,130	<4
C4 Reactor 4 outflow	24	<0.001	6	1,370	1,630	<4
CI Column In	41,000	113	10,900	26,600	7,810	<4
CO Column Out	75	<0.001	5	<1	10	15
CM Crystal Mine	19,000	1,280	19,700	53,000	15,300	405